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Fluid regulation and time course of erythropoietin during multifactorial strain of Austrian Special Forces survival training

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Abstract

The aim of this study was to provide data on fluid-regulating mechanisms with special regard to the role of plasma proteins in the control of plasma volume (PV), and to investigate erythropoietin production and release during a period of prolonged multifactorial strain. 29 male subjects, with a mean age of 22.2±2.8 years, were studied during a 5 day lasting survival training including restricted water (1| $H_2O \cdot day^{-1}$) and food intake (628 $kJ \cdot day^{-1}$) additionally to physical exercise and sleep deprivation (20 h within 5 days). Under field conditions heart rate was monitored continuously, and body mass, body composition and blood parameters were measured at (T1), after 72 h (T2), after 120 h (T3) and in the recovery period after 48 h (T4) and 72 h (T5). The estimated energy expenditure was approximately 24000 kJ day^{-1}. The mean decrease of body mass was 6.77 kg (9.5%) at T3 ($p<0.001$). A reduction of total body water of 3.8| was estimated at T3. Serum creatinine ([Cr]) was raised at T3 by 18.5% ($p<0.0001$). The PV decreased by 3.7% ($p<0.0001$) at T2, increased by 1.6% ($p<0.0001$) at T3 and was not different to baseline at T4 (+0.2%; n.s.). Plasma proteins shifted into the intravascular space at T2 and T3 and moved out of the intravascular space at T4 and T5. Our data provide evidence that this mechanism assists PV-homeostasis efficiently over a period of 120 h even under conditions with a fluid loss of almost 8% of the total body water. EPO controls at T1 were 15.2 ± 8.8 $mU.ml^{-1}$. EPO was decreased during the course (T2: 8.7 ± 7.9 $mU.ml^{-1}$; $p<0.01$ and T3: 11.6 ± 6.7 $mU.ml^{-1}$; $p<0.01$) and showed a significant increase in the recovery period. Serum iron increased from 13.5 ± 4.5 µ $mol.l^{-1}$ at T1 to 24.5 ± 4.1 $\mu mol.l^{-1}$ at T2 ($P<0.01$) and decreased during recovery. Haptoglobin (HAPTO) decreased from 165.4 ± 55.3 $mg.dl^{-1}$ at T1 to 85.8 ± 51.7 $mg.dl^{-1}$ at T3 ($P<0.01$). Thereafter HAPTO increased (T4 132.0 ± 52.2 $mg.dl^{-1}$, $P<0.01$) and remained below control level at T5 (131.6 ± 58.3 $mg.dl^{-1}$, $P<0.01$). Transferrin decreased continuously from 303.3 ± 65.3 mg . dl^{-1} at T1 to 256.8 ± 58.8 $mg.dl^{-1}$ at T5 ($P<0.01$). Ferritin increased from 70.2 ± 50.2 $ng.ml^{-1}$ at T1 to and 150.1 ± 60.2 at T3 ($P<0.01$) and returned to control level at T5 (85.7 ± 44.8 $ng.ml^{-1}$, $P<0.01$). [Hb] increased from T1 (15.3 ± 0.7 $g.dl^{-1}$) to T2 (16.6 ± 0.7 $g.dl^{-1}$, $P<0.01$) and decreased significantly thereafter (T5 14.6 ± 0.6 $g.dl^{-1}$, ($P<0.01$). Haematocrit increased from 44.2 ± 2.1 % (T1) to 46.8 ± 1.9 %, $P<0.01$) at T2 and remained afterwards below the control (T5 41.4 ± 1.8 %, $P<0.01$). It seems that EPO production and release is diminished by nutritional factors, i.e. mainly caloric intake, during prolonged physical strain. In the recovery period a rapid EPO increase took place to normalise red cell mass. These data shade new light upon the changes of erythropoiesis in astronauts observed during and after space flight. Since astronauts also show body mass losses and decreased EPO levels in a similar range during and immediately after space flights the question arises whether this might be due to a lowered caloric and/or protein intake rather than an effect of micro-gravity per se.

Introduction

Several kinds of sustained military operations with prolonged exercise can lead to a negative energy balance (16) and can induce dehydration even under ad libitum fluid intake (27,28,40). This study addressed specific questions posed to us by the Command of the Austrian Special Forces due to the fact that their survival training is designed to create multifactorial stress to evaluate the trainees under physically and psychologically demanding conditions. The course schedule included fluid restriction and food deprivation combined with prolonged exercise. Long-term physical exercise is known to have strong influences on the fluid-regulating mechanisms of the human organism which can endanger the size of the plasma volume (PV) and the extracellular sodium concentration. Beside fluid regulating hormones (2), the control of PV depends on

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plasma protein kinetic (21,23,33). In most studies dealing with long-term physical exercise, a decrease of PV was found during or immediately after exercise (2,13,19,18,33). It is still unclear to what extent such exercises lead to changes of PV, thus, being haemoconcentration at least partly responsible for the increase of various parameters of metabolism. The first aim of the present study was to investigate the time course of PV and the influence of plasma proteins as regulators of PV.

Since short-term protein deprivation can impair erythropoiesis in healthy individuals (10) and a severe negative energy balance occurs during food deprivation of the five day lasting survival training of the Austrian Special Forces the second aim of the field study reported here was designed to investigate the time course of serum EPO and related haematological parameters.

The data of the present report are currently under evaluation of reviewed journals and preliminary results were partly presented at the international symposium on "The Physiology and Pathophysiology of Exercise Tolerance", September 1994 in Ulm, Germany (41). This study received a grant of the Austrian Bundesministerium für Landesverteidigung, BMLV ZL.65.505/43-5.2/92 and of the German Bundesministerium für Forschung und Technik, BMFT (DARA) 01QV8712-50QV87120.

Subjects and Methods

The subjects were participants of a ranger training unit of the Austrian Army Special Forces. The data of 29 subjects (22.2±2.8 years; range 18-28 years) were collected during a survival training course in August 1993 after informed written consent was obtained. The anthropometric data and physiological variables of the subjects before the course are shown in Table 1.

Table 1. Anthropometric data and physiological variables of the subjects

n=29	Mean	SD	Range
Age (years)	22.2	2.8	18 – 28
Heigh (cm)	178.0	6.3	162 – 193
Body mass (kg)	73.5	8.6	60 – 96
Lean body mass (kg)	64.4	7.4	52 – 86
Body fat (kg)	10.6	2.4	7 – 16
Total body water(l)	48.0	4.9	40 – 63
HR at rest (beats· min^{-1})	53.5	1.6	38 – 60
$HR_{max,CE}$ (beats· min^{-1})	186.3	10.4	162 – 207
Relative maximal work capacity (W· kg^{-1})	4.5	0.46	3.75 – 5.71
Relative $\dot{V}O_{2max}$ (ml· min^{-1}· kg^{-1})	52.7	5.55	43.8 – 67.3

Protocol: After a laboratory testing program to determine anthropometric data, health status and physical performance, the subjects abstained from strenuous physical work in the 24 h-period before the course but had performed physically strenuous military training during the preceding weeks.

The five day survival training course took place in a woody area 430 m to 570 m above mean sea level. Meteorological data were measured at 0600 hours, 1200 hours, 1800 hours and 2400 hours. The mean temperature was 19.9 (SD4.4)° C with a range from 10.1° C to 28.6° C. The relative humidity was 61.1 (SD19.7) % with a range from 35% to 95% and the sky was clear. The wind speed was 3.0 (SD2.2) m· s^{-1} with a range from 1.1 m· s^{-1} to 12.0 m· s^{-1}. During a period of 120 h the subjects had to perform 90 km of marching, partly with tactical missions, during which 22.3 (SD 3.7) kg of clothes and military equipment had to be carried around. The subjects slept only 20 h without tent and sleeping bag. They daily received 1l of water and additionally approximately 1l in the morning of the first day and 1l in the afternoon of the fourth day. After a breakfast on the first day of about 6250 kJ (1500kcal) the mean food intake during the five day course

was only 628 kJ· day^{-1} (150 kcal· day^{-1}). Body mass, body composition, tissue thickness and blood parameters were measured early in the morning on day 1 before the course started (T1), after 72 h (T2), after 120 h at the end of the course (T3) and in the recovery period after 48 h (T4) and 72 h (T5). Food and fluid intake in the recovery period were ad libitum and could not be controlled.

Physical work capacity: The maximal oxygen uptake ($\dot{V}O_{2max}$) was estimated for each subject from the maximal work achieved during cycle ergometry (CE) as described in detail in a previous report (42).

Monitoring of heart rate: The method has already been described in detail previously (42). Briefly, the heart rate (HR) was monitored continuously with a sport-tester PE 4000 (Polar Electro, Kempele, Finland) in representative subgroups of subjects and was based on beat-by-beat ECG measurement with HR transmission by telemetry. From the HR - readings the mean intensity of the physical activity during the course was calculated as a percentage of $HR_{max,CE}$ ($\%HR_{max,CE}$). Around 80% of the physical activity during 120 h was Δ50% of the $HR_{max,CE}$. During the last 12 h of the course until T3 (marching at night) the subjects had a mean HR around 50% of the $HR_{max,CE}$. The energy expenditure, estimated by analysing HR-recordings, and calculations by references for energy expenditure during physical work (37), was approximately 24000 kJ· day^{-1} (5760 kcal· day^{-1}).

Body composition was measured at T1 by a bio-electrical impedance analysis (5) using a BIA 101- S analyser (RJL, Detroit, Mich., USA). The values are shown in Table 1.

Blood samples were drawn by repetitive venipuncture with a 20-G needle from different cubital veins in the same sitting position with identical positioning of the arm at T1,T2,T3,T4 and T5. All samples were transported immediately to the laboratory after collection. Metabolic and haematological parameters were analysed within 3 hours after collection. For all other parameters serum or plasma was obtained from whole blood in a centrifuge and stored at Δ30°C until analysis within 2 weeks. Haematocrit (Hct) and haemoglobin concentration ([Hb]) were measured with an auto-sampler T890 (Coulter Electronics, Luton, England). The intra-assay coefficient of variability (CV) was <1.5% (n=20) for these methods. The serum concentrations of uric acid ([UA]; CV<2.0%) and blood urea nitrogen ([BUN]; CV<1.3%) were determined enzymatically, total protein ([TP]; CV<2.6%) using the biuret method, and creatinine ([Cr]; CV<2.7%) by the Jaffé reaction, employing an BM/Hitachi 747 *spectrophotometer* (Boehringer Mannheim GmbH, Mannheim, Germany). The sodium concentration ($[Na^+]$) was analysed from serum by flame-photometry (KLiNa, Beckman). The intra-assay CV was <1%. The colloid osmotic pressure (COP) was measured with an BMT-921-onkometer (Thomae, Germany), the CV was 0.44%. Plasma osmolality (Osm_{pl}) was measured by freezing-point depression by a digital micro-osmometer type 5B (Roebling Company, Berlin, Germany). The CV was 0.6%.
EPO was measured by using a commercial available ELISA (IBL, Hamburg, Germany). The coefficient of variation (CV) for this method was 4.8 %.
Serum iron ($[Fe^{++}]$) was measured with atomic absorption spectrophotometry (Philips SP9) without dilution (CV <1%). Ferritin (FER) was analysed with a commercial available IRMA (Company Bio-Rad) (CV 3.4%).
Haptoglobin concentration (HAPTO; CV<3%) and Transferrin (TRANS; CV <5%) was measured by nephelotomy (Nephelometer 100, Behring Werke, Germany). HAPTO was measured to estimate haemoglobin loss by intravascular haemolysis. The values are shown in Table 1. Based on the stoechiometric relation between haemoglobin and HAPTO the haemoglobin consumption by haemolysis was calculated according to the literature (35). Assuming a PV of approximately 3.5l, the total intravascular [Hb] loss via haemolysis was 1,8g. This cannot account for relevant changes of [Hb] and Hct in the calculation of PV.
The percentage changes in plasma volume (%ΔPV) were calculated from [Hb] and Hct according to the equation given by Strauss et al. 1951 (38).
To obtain more information about the maintenance of the colloid osmotic capacity by plasma proteins it was necessary to consider changes of [TP] and PV simultaneously. Of the two it is possible to appraise the change of the intravascular plasma protein mass ($IVTP_{pl}M$), which is mostly responsible for the water-binding capacity of the intravascular space. As described previously (33) we related the percentual changes of [TP] (%Δ[TP]) to the percentual changes of PV (%ΔPV) by calculating the difference of % ΔPV and % Δ[TP]. This difference describes the relationship between % Δ [TP] predicted by % ΔPV compared to measured % Δ[TP]. If there is no increase or decrease of $IVTP_{pl}M$, the expected value (E) of the above-mentioned difference is not

significantly different from zero (E=0). This can be interpreted as pure haemoconcentration or haemodilution. A significant value E>0 can be interpreted as a gain of $IVTP_{pl}M$ into the vascular space, whereas a significant value E<0 can be interpreted as a loss of $IVTP_{pl}M$ out of the vascular space.

Statistics

The data showed normal distribution determined by Chi Square test and Kolmogorov-Smirnov test. The hypothesis of differences in repeated measures was tested by t-test. The Bonferoni technique was used to protect significance level. Interaction effects between variables were tested by ANOVA. Dependencies between variables were tested by linear regression. The results are presented as mean ΔSD. If not otherwise indicated changes of percentage values are presented as the means of individual, percent changes. Statistical significance was attributed if the probability of error was less than 5% ($p<0.05$).

Results

1. Time course of body mass

The observed decrease of mean body mass was 5.12 kg (6.8% ; $p<0.001$) at T2, 6.77 kg (9.5%; $p<0.001$) at T3, 0.95 kg (1,3%; $p<0.05$) at T4 and 0.68 kg (0.9%; n.s.) at T5. The decrease of the body mass was not correlated to the baseline values of physical work capacity, body mass, total body water, body fat, or weight of clothes and military equipment which had to be carried around by the subjects.

2. Time course of parameters measured from blood samples (Table 2)

$[Na^+]$ was within normal range over the whole time course, though it was slightly decreased at T2 by 2.24 $mMol \cdot l^{-1}$ ($p<0.05$) and at T5 by 3.0 $mMol \cdot l^{-1}$ ($p<0.01$) compared to T1. Osm_{pl} was within normal range and did not change over the whole time course. COP (Table 2) was increased at T2 and T3 and decreased at T4 and T5 compared to T1.

[TP] had increased by 11.7% ($p<0.0001$) at T2, 2.6% ($p<0.01$) at T3 and was decreased ($p< 0.0001$) at T4 (8.2%) and T5 (5.7%). The changes of the [TP] at T2 (Fig. 2) and T3 (Fig. 3) correlated with the changes of COP .

[BUN] and [UA] were increased ($p<0.0001$) at T2 by 57.3% and 48.8%, and at T3 by 98.7% and 88.6%. At T4 [BUN] and [UA] had already returned to values within normal range. No correlations or dependencies were found between [BUN] and [UA] and the changes of PV.

[Cr] was increased ($p<0.0001$) at T3 by 18.5% compared to baseline values, and was not changed significantly at any other time. No correlation or dependency was found between [Cr] and changes of the PV, [BUN] or [UA].

Table 2. Time course of parameters measured from blood samples.
T1= before the course, T2= after 72 h of strain, T3= end of the course after 120 h of strain, T4= 48 h of recovery, T5= 72 h of recovery. Not significant (ns) changes are indicated. All other changes of parameters were significantly different to the initial value at T1 by at least $p<0.05$. Abbreviations for demonstrated parameters are explained under methods.

n=29	T1		T2		T3		T4		T5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$[Na^+]$ (mmol· l^{-1})	138.2	4.2	136.0	3.6	^{ns}139.0	5.4	^{ns}137.9	3.5	135.2	2.1
Osm_{pl} (mmol· l^{-1})	290.1	5.8	^{ns}289.7	7.4	^{ns}290.2	5.1	^{ns}286.6	11.7	^{ns}290.9	6.1
COP (mmHg)	26.8	2.0	32.7	2.2	30.2	2.2	23.7	2.1	24.0	2.8
[TP] (mg· dl^{-1})	73.2	3.4	81.7	4.9	75.1	3.9	67.2	3.4	69.0	2.4
[BUN] (mg· dl^{-1})	15.3	2.9	23.6	3.7	29.4	3.7	^{ns}16.3	2.8	^{ns}16.3	2.9
[UA] (mg· dl^{-1})	5.5	0.9	8.1	1.0	10.3	1.3	5.2	0.8	5.3	1.0
[Cr] (mg· dl^{-1})	1.09	0.10	^{ns}1.09	0.10	1.29	0.11	^{ns}1.11	0.07	^{ns}1.09	0.09

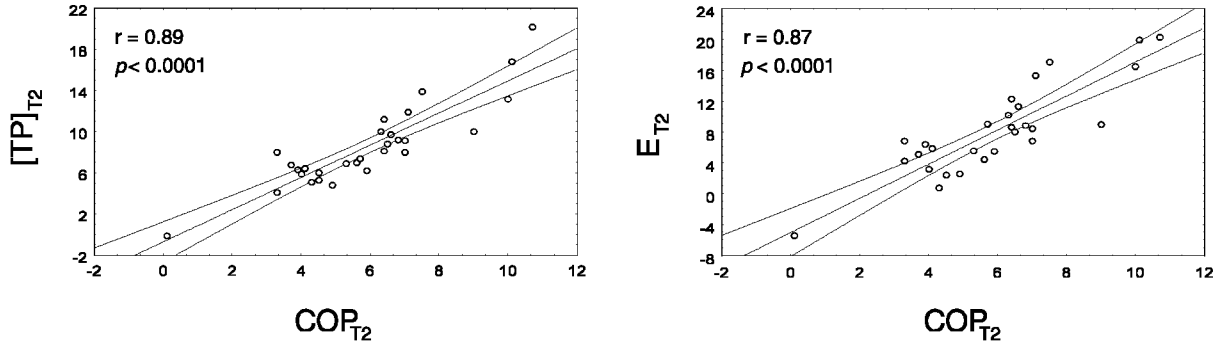

Fig. 1. Relationship between changes after 72 h (T2) of strain. Colloid osmotic pressure (COP) vs total protein concentration ([TP]) and COP vs the E-values. The definition for E-values, which indicate protein shifts, is given under methods.

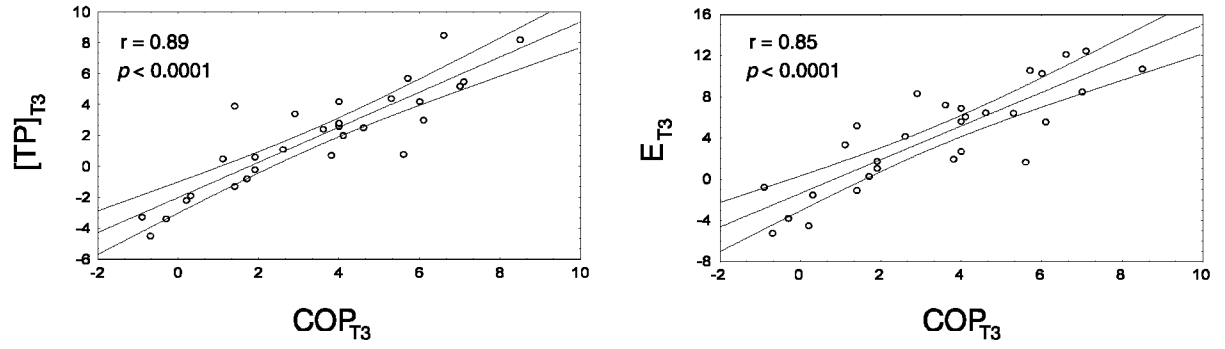

Fig. 2. Relationship between changes after 120h (T3). For definitions see Fig.1

3. Percentage changes of PV and protein shifts (Fig.3)

The level of statistical significance of the changes is shown in Fig. 3. PV was decreased at T2 by 3.7%, raised above baseline value at T3 by 1.6%, was statistically indistinguishable from baseline at T4, and was decreased at T5 by 2%. There was a significant gain of intravascular [TP] seen at T2 (E=8.0) and T3 (E=4.2). A significant loss of [TP] out of the intravascular space appeared at T4 (E= Δ8.4) and T5 (E= Δ7.7). Between COP and the E-values a significant correlation was found at T2 (Fig.1) and at T3 (Fig.2). No correlations or dependencies were found between E and $[Na^+]$.

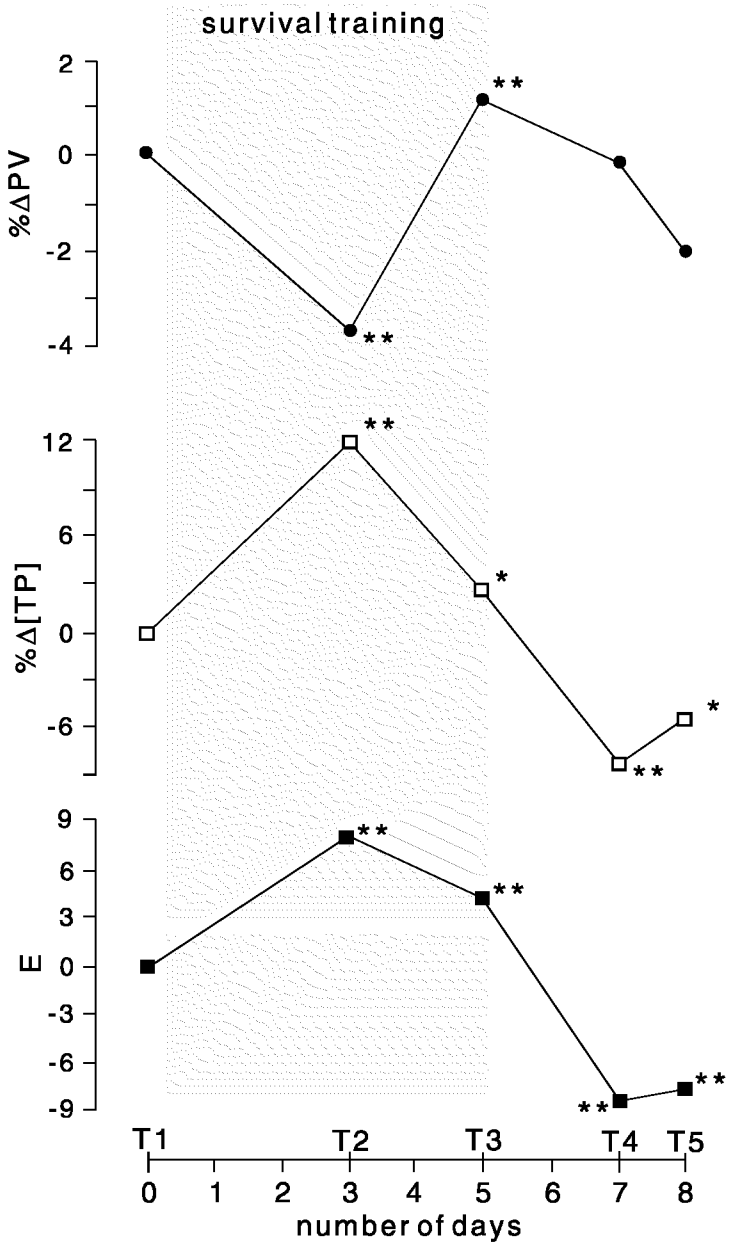

Fig. 3. Time course of percentage changes of plasma volume (%ΔPV), percentage changes of total protein concentration (%Δ[TP]) and the mean E-values (a detailed definition of E-values is given under methods) before, during and after one week with food and fluid deprivation. A significant value E>0 can be interpreted as a gain of the intravascular total protein mass into the vascular space, whereas a significant value E<0 can be interpreted as a loss of the intravascular total protein mass out of the vascular space. The level of significance of either parameter in relation to the initial value was: * p<0.05; ** p<0.0001

4. Serum EPO and related haematological parameters

The main results are summarised in figure 4,5 and 6.

EPO controls at T1 were 15.2 ± 8.8 mU· ml^{-1}, T2 8.7 ± 7.9 mU· ml^{-1} (P<0.01), T3 11.6 ± 6.7 mU· ml^{-1} (P<0.01), T4 23.4 ± 12.0 mU· ml^{-1} (P<0.01) and at T5 18.7 ± 11.3 mU· ml^{-1} (P<0.05).

[Fe^{++}] increased from 13.5 ± 4.5 µmol· l^{-1} at T1 to 24.5 ± 4.1µmol· l^{-1} at T2 (P<0.01), decreased at T3 20.5 ± 6.7 µmol· l^{-1} (P<0.01) and T4 14.5 ± 4.4 µmol· l^{-1} and increased towards T5 again (17.0 ± 5.4 µmol· l^{-1}, P<0.01).

HAPTO decreased from 165.4 ± 55.3 mg· dl^{-1} at T1 to 117.4 ± 55.5 mg· dl^{-1} at T2 (P<0.01) and 85.8 ± 51.7 mg· dl^{-1} at T3 (P<0.01). Thereafter HAPTO increased (T4 = 132.0 ± 52.2 mg· dl^{-1}, P<0.01) and remained below control level at T5 (131.6 ± 58.3 mg· dl^{-1}, P<0.01).

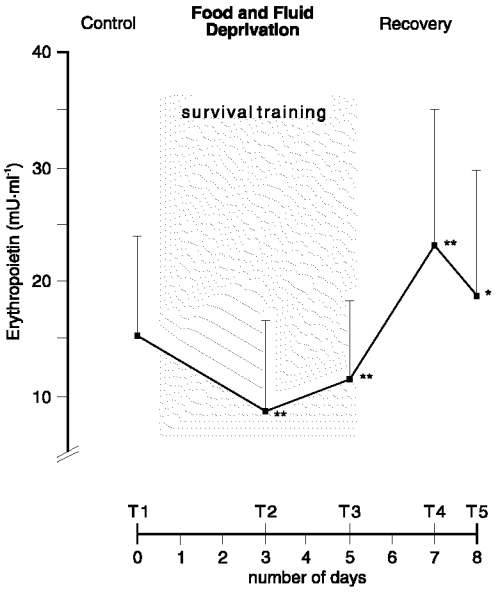

Fig. 4. Time course of erythropoietin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: *P<0.05, **P<0.01.

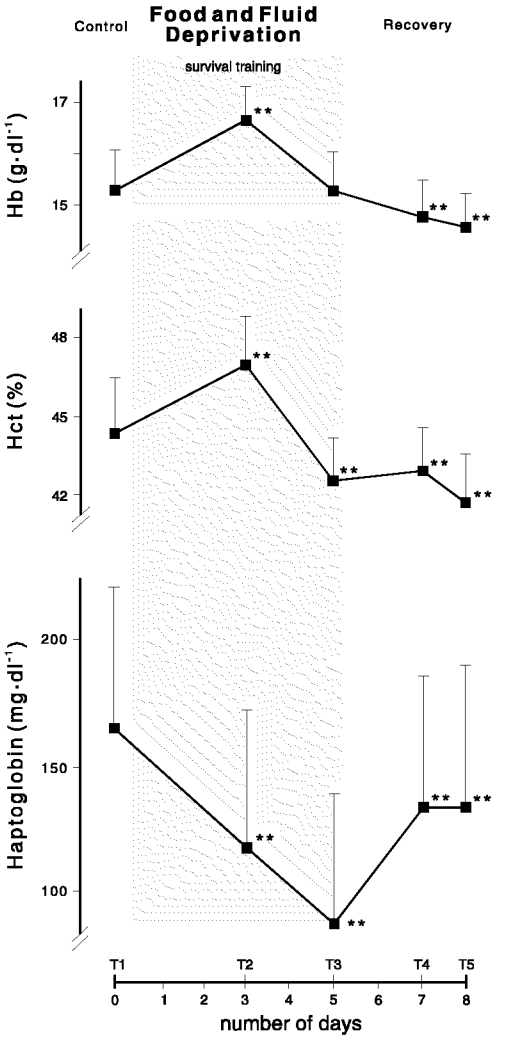

Fig. 5. Time course of haemoglobin (Hb), haematocrit (Hct) and haptoglobin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: **P<0.01.

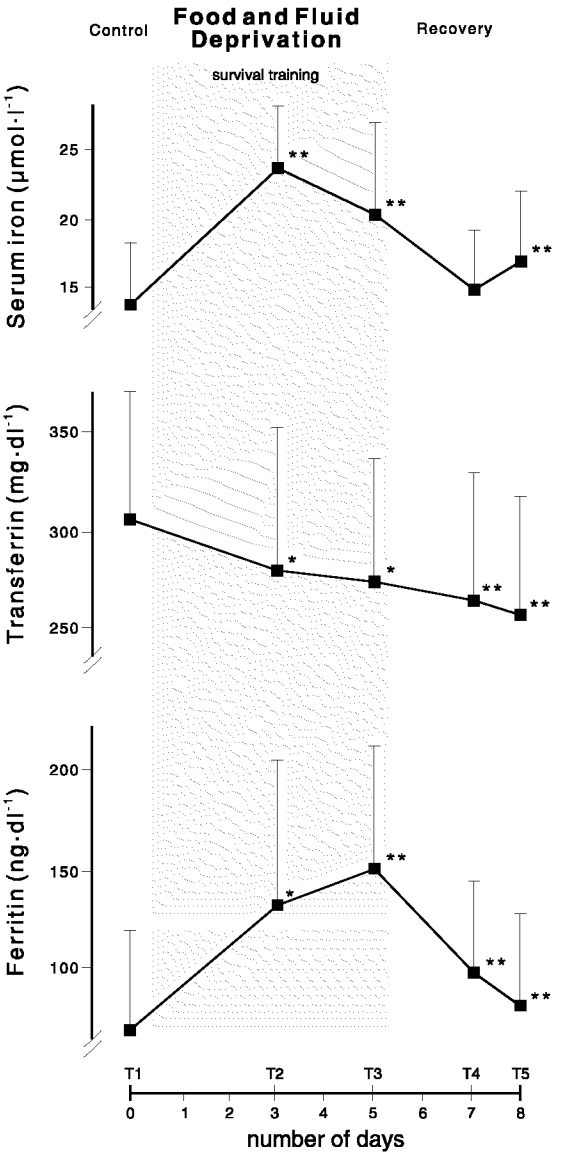

Fig. 6. Time course of serum iron, transferrin, and ferritin before, during and after one week with food and fluid deprivation. The level of significance of either parameter in relation to the initial value was: *P<0.05, **P<0.01.

TRANS decreased continuously over the week from 303.3 ± 65.3 mg· dl^{-1} at T1 to 272.4 ± 63.1 mg· dl^{-1} at T3 (P<0.05) and 256.8 ± 58.8 mg· dl^{-1} at T5 (P<0.01).
FER increased from 70.2 ± 50.2 ng· ml^{-1} at T1 to 134.4 ± 70.4 ng· ml^{-1} at T2 (P<0.01) and 150.1 ± 60.2 at T3. Thereafter FER decreased (T4 101.7 ± 45.1 ng· ml^{-1}, P<0.01) and reached control level at T5 (85.7 ± 44.8 ng· ml^{-1}, P<0.01).
[Hb] increased slightly from T1 (15.3 ± 0.7 g· dl^{-1}) to T2 (16.6 ± 0.7 g· dl^{-1}, P<0.01), showed no significant changes compared to the controls at T3 (15.2 ± 0.7 g· dl^{-1}), and decreased significantly at T4 (14.8 ± 0.6 g· dl^{-1}) and T5 (14.6 ± 0.6 g· dl^{-1}) (P<0.01).

Discussion

From the HR-monitoring and calculations (37) based on the pattern of the physical activity it can be estimated that the average intensity of exercise was 35% to 40% of the $\dot{V}O_{2max}$. The calculations of energy expenditure during the 120 h of strain leads to an estimation of approximately 3 kg loss of body mass by catabolism.

The mean decrease of body mass at the end of the course was 6.77 kg, which was partly due to fluid loss. It was estimated that at this time a reduction of total body water by 3.8| had occurred.

According to prior results under comparable conditions (28), although confounded in the present study by a restriction of fluid intake, it could be observed that the extracellular [Na^+] was not altered in a biologically relevant amount. Although the [Na^+] was within normal range over the whole time course, there was a slight decrease of 2.6% after 72h of exercise. This was in parallel with a slight decrease of PV. It can be assumed that the mechanisms responsible for water retention and production of increased plasma solute concentration during exercise have not been activated sufficiently at this time to counterbalance completely the net water loss. Convertino et al.(11) described a PV decrease of > 3.7 % as one of the threshold stimuli to produce increased plasma solute concentration during exercise. After 120 h the [Na^+] was unchanged whereas PV was 1.6% above baseline values. The slight increase above base line values of PV after 120 h is in accordance with a prior report of seven consecutive days of hill-walking (40). Williams et al. (40) assumed, that the mechanisms responsible for water retention took longer than one day to become fully operative, and subsequently more than counterbalanced the net water loss. The present study could show, that the PV can be maintained over a period of several days of exercise even under conditions with a net fluid deficit of almost 4| .

During the course [TP] was markedly higher than predicted by ΔPV. An E-value of +8 after 72 h and +4.2 after 120 h indicated a significant shift of plasma proteins into the intravascular space during the period of exercise, similar to changes found immediately after a marathon run (33). Additionally to previous studies we found a significant relationship between COP and [TP] after 72 h and 120 h of strain. If this relationship had been based only upon haemoconcentration, ΔCOP and %ΔPV should have been correlated. This was not the case, but COP and the E values correlated significantly at 72 h and after 120 h, indicating that changes of the $IVTP_{pl}M$ lead to the changes in COP.

These results supported the hypothesis that one important factor for the maintenance of PV during prolonged exercise is provided, beside other complex regulating mechanisms (7), by protein shifts from the extravascular into the intravascular space (4,33).
In a critical evaluation of the used formula given by Strauss (38) for the calculation of PV changes reference should be made to the fact, that this formula includes Hct and [Hb]. Reliable results can only be achieved in the absence or with knowledge of the amount of intravascular haemolysis. Therefore we measured HAPTO providing an objective marker for intravascular haemolysis, which occurred only in a negligible amount in the present study (see below).

The elevated values of [BUN] and [UA] during the course are indicating at catabolism and reduced excretion. Ad libitum rehydration led to normal values of these parameters within 48 h. Also, the highly significant increased [Cr] at the end of the course was not due to haemoconcentration. This could be proved by an increased PV of 1.6% at this time and by the fact that [Cr] showed no statistical relationship (regression analysis, Student´s *t*-test, ANOVA) with [BUN] and [UA]. Thus, this increase could be interpreted as an independent factor indicating at a reduced renal excretion of creatinine. This points out a reduced creatinine clearance which correlates with a decrease in urine flow mainly due to a reduction in glomerular filtration rate (9).

The most prominent findings of the second question addressed in the present study were the decreased EPO levels during survival training and a rapid increase post exercise (Fig. 4). HAPTO concentrations decreased during and increased after the survival training (Fig. 5), TRANS decreased continuously (Fig. 6). [Fe^{++}], [Hb] concentrations and Hct were inversively related to EPO concentration. Data on the EPO response during comparable conditions of prolonged physical strain are scanty available in the literature. Most likely is the study done by Lindemann et al. (24) who measured haematological changes during a Norwegian combat course. Some of the EPO related haematological parameters ([Fe^{++}], HAPTO, TRANS) in this study behaved similar to our findings but Lindemann et al. (24) found in contrast to our findings an increased erythropoietic activity during the course. Unfortunately, at that time only pooled plasma from a group of subjects could be used and that method cannot serve reliably as a reference anymore. De Paoli Vitali et al. (14) measured EPO

concentrations in 11 well-trained athletes before and immediately after a 50-km cross-country ski race. They found an increase immediately after the race. Vedovato et al. (39) analysed EPO concentrations in 18 athletes before and immediately just after a 20-km long-distance run. They found as well a marked increase in EPO concentrations. Schwandt et al. (36) analysed the influence of prolonged physical exercise in marathon runners on EPO concentration before and several hours after a race. It was found that EPO values increased significantly 3 hrs, and more impressively 31 hrs after the race.

It is well-known that [Hb] and iron stores are affected by physical exercise which might have influence on a later stimulation of EPO, therefore these data were of special interest. Our soldiers showed normal [Hb] and FER concentration at T1. The [Hb] remained rather stable during the course, which is in contrast to the findings of Lindemann et al. (24). In our study HAPTO decreased from 165.4 ± 55.3 $mg \cdot dl^{-1}$ at T1 to 85.8 ± 51.7 $mg \cdot dl^{-1}$ at T3 and the mean plasma volume was approximately 3.5 l. Then the [Hb] loss by haemolysis would have been only 1.83 g haemoglobin. Thus, it could be shown, that haemolysis occurred only in a negligible amount. This finding can be explained by the fact, that all marching exercise had to be performed in a terrain with soft ground, wearing special combat boots with soft and thick rubber soles.

On the other hand, the biochemical constellation of an increased serum [Fe^{++}], a decreased TRANS and an increased FER is a typical sign for a haemolytic anaemia with impaired erythropoiesis (20) which would be in line with decreased EPO values found during the survival training program. However, the interpretation of the given data is complicated by the fact that while Ferritin is a form of storage protein for iron, it is also an acute phase reactant (12). As pointed out by Moore et al. (25) some acute phase-like-disturbances may occur as transient normal adaptations to the multifactorial stress of comparable military conditions. Thus, the serum concentration of Ferritin can rise sharply in the absence of any change in total body iron stores. The classical biochemical constellation of an haemolytic anaemia might be misleading under the described conditions.

The body mass changes found in our group are similar to those of astronauts. Astronauts usually show after space flights lasting longer than one week a body mass loss of about 7% and sometimes a negative inflight nitrogen balance up to -4.5 $g \cdot day^{-1}$ as it is known from the six crewmembers of the first two Skylab Missions (23,26). Furthermore, the astronauts regularly show a decreased red cell mass (26) and decreased EPO levels inflight whereas postflight a rapid increase of EPO production and release was observed (22). These results were recently confirmed by EPO data from one cosmonaut of the German MIR´92 Space Mission (32) and from four astronauts of the German D-2 Space Mission (17).

The reason for the decreased EPO level is not yet known. It is interesting to note that the time course and extent of the EPO response are similar to findings of the present group. The multifactorial strain of work (except the physical work load) of the astronauts of the German D-2 Space Mission was comparable to the present group. It is known that especially during the first days under micro-g-conditions vomiting occurs and food and fluid intake are usually reduced. It might well be that the reduced EPO production observed during space flights is influenced by an overall lack of caloric and/or protein intake, which was described for astronauts and cosmonauts in the literature (26). This conclusion is in line with the findings of Caro et al. (8) and Rosenberg et al. (34). Caro et al. (8) investigated the effect of the thyroid hormone T3 and glucose supplementation on EPO production in rats and found that a 48 h fasting significantly reduced the circulating levels of thyroid hormones and the production of renal and extrarenal EPO in response to hypoxia. In their opinion the caloric deprivation is primarily responsible for the decreased EPO levels induced by fasting in rats and that this effect is probably mediated by a decreased level of T3 and a decreased responsiveness to it. Although in contrast, Jelkmann et al. (19) who investigated the effects of fasting on EPO production in rats found that a reduced food intake cannot account for the fall in EPO during continuous hypoxia. However, in the present study near sea-level (430-570 m altitude) lowered T3 levels in the subjects can be expected as shown by Aakvaag et al. (1). Furthermore, Opstad and Aakvaag (29) investigated under comparable conditions of strain well-fed subjects and a group of soldiers with food deprivation, revealing significantly higher levels of T3 in the well-fed subjects. It is interesting to note that those subjects of our group who had the most impressive EPO increase during the recovery period reported that they had eaten mainly sweets, cakes, carbohydrates during the recovery period. This observation would support the results from Caro et al. (8) that it is mainly the caloric intake which influences the responsiveness of the erythropoietic system during food deprivation. These findings are not in line with those studies in rats and man from Bethard et al. (6),

Reissmann (31), Anagnostou et al. (3), Catchatourian et al. (10) and Rosenberg et al. (34) who concluded that protein intake is more essential for maintenance of normal erythropoiesis than total caloric intake.

Concluding Remarks

1. The results of the present study provide evidence that, physical exercise of moderate intensity lasting 120 h confounded by food deprivation and a fluid loss of almost 8% of the total body water did not change PV in a biologically relevant amount. Important counterregulatory mechanisms were, at least in part, protein shifts into the intravascular space, accompanied by a potentially dangerous reduction of the glomerular filtration rate. This result of the study already lead to a cancellation of the fluid restriction during survival training of the Austrian Army ranger course.

2. The knowledge of the mechanisms behind EPO production and release under terrestrial conditions is not only essential for the humans' well-being during and after prolonged physical strain with food and fluid deprivation on earth but as well as for astronauts' long-term space flights, i.e. to develop effective countermeasures against the "astronauts' anaemia" and a general understanding of the factors being relevant for EPO control under physiological and pathophysiological conditions.

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